Membrane protein biogenesis by the EMC

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The endoplasmic reticulum (ER) membrane protein complex (EMC) was identified over a decade ago in a genetic screen for ER protein homeostasis. The EMC inserts transmembrane domains (TMDs) with limited hydrophobicity. Two recent cryo-EM structures, and a third model based on partial high- and low-resolution structures, suggest how this is accomplished.

Insertion of strongly-hydrophobic TMDs into the ER membrane is mediated by the Sec61 complex for co-translational insertion and the GET complex for posttranslational insertion of tail-anchors (Volkmar and Christianson, 2020). By contrast, the EMC inserts TMDs of limited hydrophobicity, frequently located at the N- or C-termini of proteins, and is involved in biogenesis of multi-spanning membrane proteins (Volkmar and Christianson, 2020).

The EMC is highly conserved (Wideman, 2015). In vertebrates, ten subunits have been identified (EMC1-10), two of which, EMC8 and EMC9, are homologous and the result of a vertebrate-specific gene duplication (Wideman, 2015). In *S. cerevisiae*, EMC8 has been lost (Wideman, 2015). Only EMC3 displays clear homology to other membrane protein insertases, the Oxa1 family (Volkmar and Christianson, 2020; Wideman, 2015). This family includes YidC, which inserts TMDs into the bacterial cytoplasmic membrane, usually in cooperation with the Sec61-homologous SecYEG channel (Volkmar and Christianson, 2020). Their association, along with the SecDF ancillary complex, forms a holo-translocon capable of protein secretion and TMD insertion, with striking similarities to the EMC complex (Martin et al., 2019).

Recent work by Pleiner *et al.* presented a 3.4Å cryo-EM structure of the human EMC purified *via* a GFP-tag on EMC2 and incorporated into a phospholipid nanodisc (Pleiner et al., 2020). The complex is formed by nine proteins (EMC1-8, EMC10) (Pleiner et al., 2020). EMC8 and EMC9 are structurally similar and their association with EMC2 is mutually exclusive (Martin et al., 2019; O'Donnell et al., 2020). Of the twelve TMDs, nine constitute the pseudosymmetric central ordered core, with a basket-shaped cytosolic vestibule formed primarily by alpha-helices of the EMC3 and EMC6 TMDs and cytosolic EMC2 (Fig. 1a) (Pleiner et al., 2020). The L-shaped lumenal domain of the EMC consists mostly of beta-sheets (Fig. 1a) (Pleiner et al., 2020), flanked by a conspicuous and conserved amphipathic alpha-helix of EMC1 sealing the vestibule at the interface between the membrane and the ER lumen, together with another smaller amphipathic helix contributed by EMC3 (Fig. 1a) (Pleiner et al., 2020). In the ER lumen the two 8-bladed propellers of EMC1 contact six of the eight other subunits and stabilize the entire complex (Fig. 1a) (Pleiner et al., 2020). Beta-sandwiches of EMC7 and EMC10 are anchored to the EMC1 lumenal domain (Fig. 1a)

(Pleiner et al., 2020). In the cytosol, the tetratricopeptide repeat (TPR) spiral of EMC2 forms a cup underneath the partially hydrophilic vestibule in the membrane between the TMDs of EMC3 and EMC6, bridging the cytosolic ends of TMDs of EMC1, 3, and 5 (Fig. 1a) (Pleiner et al., 2020). Cytosolic EMC8 is bound to the opposite face of EMC2 (Fig. 1a).

The 3.0Å cryo-EM structure of the yeast EMC presented by Bai and colleagues shows a very similar overall organization (Bai et al., 2020). Here, purification was via a 3xFLAG-tag on EMC5, and the structure of the 8-subunit complex (without EMC8/9) was visualized in detergent solution (Bai et al., 2020). The yeast complex has twelve TMDs like the human EMC, but unlike the human structure, EMC4 in yeast has three TMDs that are clearly visible (Bai et al., 2020). They are angled in the membrane pointing away from the complex at the cytosolic end (Fig. 1a), and Bai et al. propose that TMDs of EMC4, EMC3, and EMC6 form a substrate binding pocket similar to that of YidC (Bai et al., 2020). As in the human EMC there are two amphipathic helices (EMC1, EMC3) at the membrane/lumen interface (Fig. 1a) (Bai et al., 2020). In the ER lumen, yeast EMC1 only has one 8-bladed beta-propeller, to which the beta-sandwiches of EMC7 and EMC10 are anchored (Fig. 1a) (Bai et al., 2020). In the cytosol, EMC2 bridges EMC3, 4, and 5, and its TPR repeats form a cup underneath the vestibule similar to human EMC2 (Fig. 1a) (Bai et al., 2020).

The authors propose that insertion of a partially-hydrophilic TMD by the yeast EMC is mechanistically similar to insertion by bacterial YidC (Bai et al., 2020). Yeast EMC is proposed to bind substrate between TMD2 of EMC3 and TMD2 of EMC4 in a pocket with polar and positively charged amino acids at either end and hydrophobic amino acids in the center (Fig. 1b) (Bai et al., 2020). Much has been made of a conserved positive region within the EMC complex here, present in an equivalent position also in YidC (Kumazaki et al., 2014): It is claimed to be important for the incorporation of more-hydrophilic TMDs, and

perhaps responsible for the ‘positive-inside’ orientation rule (von Heijne, 1992). Yeast and human EMC3 contain a specific R31 and R26 residue, respectively, conserved also in YidC and important for function of the EMC, as well as for YidC in Gram-positive, but interestingly not Gram-negative, bacteria (Pleiner et al., 2020; Bai et al., 2020; Chen et al., 2014). Another interesting feature, also conserved with YidC, is the flexibility of the TMDs flanking the substrate-binding pocket, critical for EMC-entry of substrates (Bai et al., 2020).

In the human EMC, methionine residues in a cytosolic loop of EMC3 act as a substrate bait (Pleiner et al., 2020). Polar and charged residues within the substrate-binding groove guide the lumenal domain across the membrane, facilitated by local membrane thinning (Pleiner et al., 2020) (Fig 1b). The positive charges within the substrate-binding site exclude signal peptides and enforce the 'positive-inside rule' (Pleiner et al., 2020; von Heijne, 1992). Flexible TMDs of EMC4, EMC7 and EMC10 forming a 'lateral gate' of the substrate-binding groove allow sampling of the bilayer by the substrate TMD (Pleiner et al., 2020). As the shortened TMDs of EMC3 and EMC6 cannot stably bind the substrate TMD, they favour its release into the bilayer (Pleiner et al., 2020). The EMC1 beta-propeller(s) may recruit additional protein maturation factors in the ER lumen (Pleiner et al., 2020; Bai et al., 2020) or bind the Sec61 channel to allow cooperation between the two insertases (Bai et al., 2020).

Arguably the most interesting feature of the EMC complex is the location of a large interior cavity with distinctive hydrophilic character, which likely aids TMD insertion (Fig. 1b). We ran a coarse-grained molecular dynamics (CG-MD) simulation of the yeast EMC structure, which highlights a profound perturbation of the phospholipid bilayer in the EMC interior cavity (Fig. 1c). Here, a deep gorge forms in the cytoplasmic leaflet of the bilayer, allowing the cavity to become flooded with water (Fig. 1c). Note the location of the lipid head

groups here (lime green), which presumably define the site of amphipathic TMD insertion. The incursion of phospholipids into the centre of the EMC complex is a feature shared by the bacterial holo-translocon (Martin et al., 2019), and perhaps by all membrane protein insertases. The shape and character of the EMC cavity presumably dictate its predisposition for less hydrophobic TMDs; it would be interesting to see whether the cavities of different insertases are similarly tailored to suit their substrates.

Figure Legend

a) Cryo-EM 3D map of the human (emdb-21929) and yeast (emdb-21587) EMC, showing front and back views with individual subunits coloured. Membrane position, obtained from the OPM database, is shown by grey discs. b) Close-up view of the EMC cavity formed by EMC3 and EMC6. Left, shown in a hydrophobicity surface pattern. Right, surface representation overlapped with the TMDs of EMC3 and EMC6. EMC4, flexible and with a gate function at the substrate binding place, is shown in pink in the yeast representation. EMC4 is not visible at the atomic EMC human structure, although is observed as a weak density at the human model, accompanied by TMs of EMC7 and EMC10 (Pleiner et al., 2020).

c) The yeast EMC following >5 μs of CG-MD simulation. The protein is shown as surface, and coloured as per Pleiner et al., 2020. The computed densities of waters and phospholipid tails and phosphates are shown as blue, yellow and lime green densities, sliced to bisect the cavity for clarity. Right, inset of the EMC cavity. Methods: CG-MD simulations were built using PDB 6WB9 in a solvated symmetric POPC/POPE/cholesterol membrane and run in the Martini forcefield as described in Martin et al., 2019. 3 μs unrestrained simulations were run,

followed by 2.5 μs backbone restrained simulation for density calculation, done using VolMap in VMD (Humphrey et al., 1996).

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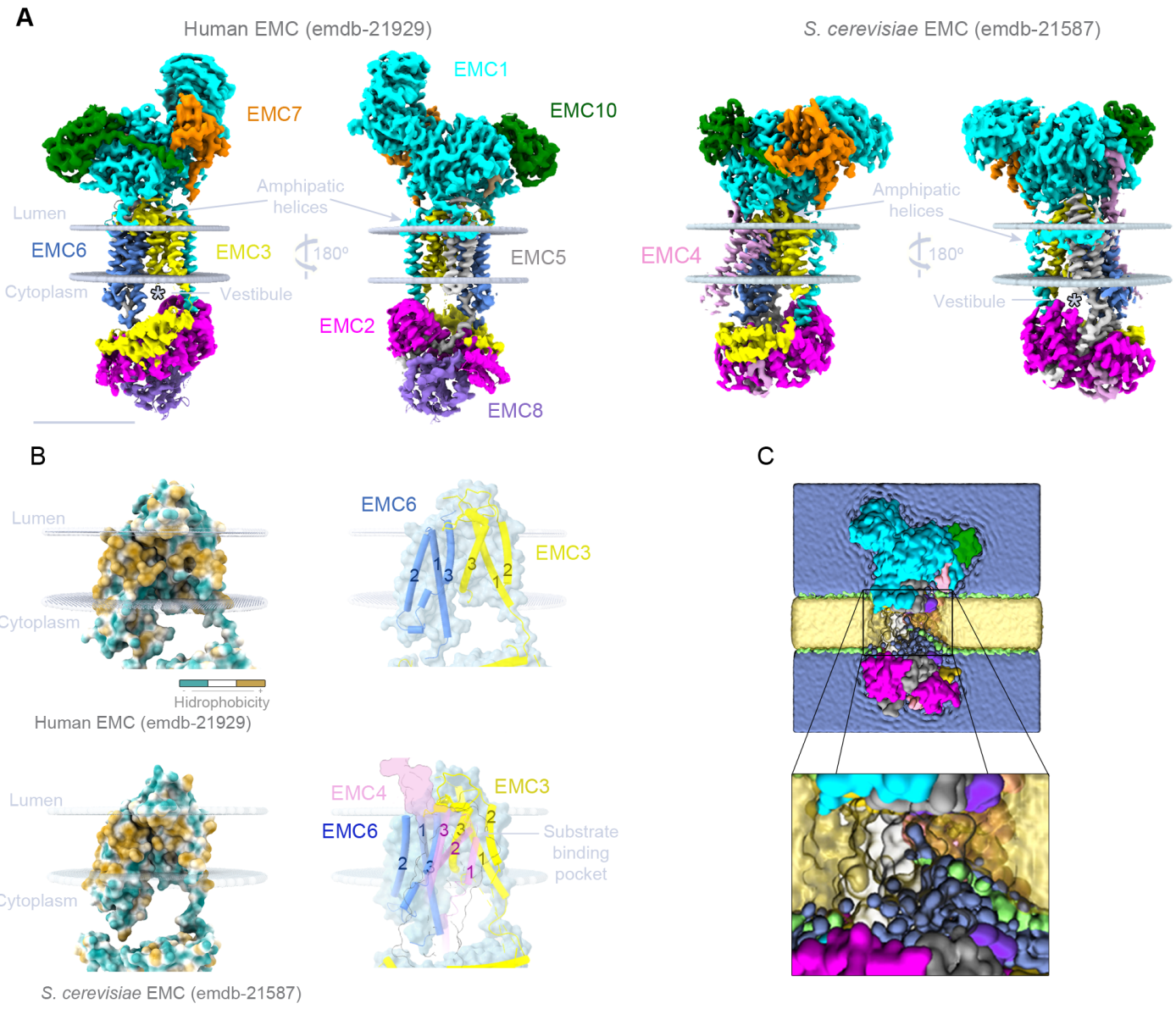
A
Human EMC (emdb-21929)
S. cerevisiae EMC (emdb-21587)
EMC1
EMC7
EMC10
Amphipatic helices
Lumen
EMC6
EMC3
180°
EMC5
Cytoplasm
Vestibule
EMC2
EMC8
EMC4
B
Lumen
Cytoplasm
Hidrophobicity
Human EMC (emdb-21929)
EMC6
EMC3
EMC4
Substrate binding pocket
S. cerevisiae EMC (emdb-21587)
C